

### **REMARKS**

Upon entry of the foregoing Amendment, Claims 7 and 11-14 are pending in the application. Claims 7 and 11-13 have been amended. Claims 8 and 10 have been canceled. New Claim 14 has been added. The amendments are supported by the specification and the original claims. No new matter has been introduced.

In the Office Action dated August 5, 2010, the Examiner sets forth a number of grounds for rejection. These grounds are addressed individually and in detail below.

#### **Specification**

The Examiner noted that pages 2 and 7 of the specification are not properly aligned. The specification has been amended to address the Examiner's concerns.

#### **Claim Rejections Under 35 U.S.C. § 112**

Claims 7-12 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the Examiner alleges that the claim do not set forth the invention as recited in the statement submitted with the IDS of 6/20/2007, which states:

The application before the U.S. Patent Office describes and claims a kit for measuring loss of vitamin D binding proteins into urine by assaying for the ability of a sample of urine to bind labeled 25-hydroxyvitamin D<sub>3</sub>. The loss of vitamin D binding proteins into urine is an indicator of salt-sensitivity (emphasis added).

Applicants would like to clarify that the phrase “loss of vitamin D binding proteins into urine” means the “excretion of vitamin D binding proteins into urine,” which results in increased concentration of vitamin D binding proteins and, consequently, higher vitamin D binding activity in the urine. In consistent with this statement, Claim 12 has been amended to recite “wherein a higher-than-normal 25-hydroxyvitamin D<sub>3</sub> binding activity in the urine is deemed indicative of salt sensitivity or predisposition to salt-associated hypertension.”

The Examiner also alleges that “it is unclear what is being claimed as if the invention is designed to be a simple test of the presence of proteins that bind 25-OHD, it is unclear why the samples are split and excess unlabeled substrate needs to be added” (page 3 of the Office Action).

Applicants respectfully submit that Claim 12, as amended, directs to a method for measuring 25-hydroxyvitamin D<sub>3</sub> binding activity in a urine sample and using the 25-hydroxyvitamin D<sub>3</sub> binding activity in the urine as an indicator for salt sensitivity or predisposition to salt-associated hypertension. Applicants further submit that a person of ordinary skill in the art would understand that the unlabeled substrate is added in a parallel binding assay to determine specific binding. The assay measures the binding of the labeled 25-hydroxyvitamin D<sub>3</sub> to a urine sample. The binding can be specific (*e.g.*, binding to the 25-hydroxyvitamin D binding protein in the urine) or non-specific (*e.g.*, binding to certain sticky materials in the urine). When an excess amount of unlabeled 25-hydroxyvitamin D<sub>3</sub> is added to the reaction, the unlabeled 25-hydroxyvitamin D<sub>3</sub> will compete with the labeled 25-hydroxyvitamin D<sub>3</sub> for specific binding but will not compete with the labeled 25-hydroxyvitamin D<sub>3</sub> for non-specific binding. Accordingly, the difference between the binding of the labeled 25-hydroxyvitamin D<sub>3</sub> alone and the binding of the labeled 25-hydroxyvitamin D<sub>3</sub> in the presence of

unlabeled 25-hydroxyvitamin D<sub>3</sub> reflect the specific binding of labeled 25-hydroxyvitamin D<sub>3</sub> in the urine sample. This type of competitive assay is commonly used and well known in the art.

The Examiner further alleges that “Applicant’s nomenclature is confusing. Applicant sometimes uses the designation “vitamin D” and sometimes “vitamin D<sub>3</sub>” seemingly interchangeably and it is unclear what is desired” (page 3 of the Office Action). Applicants respectively submit that “vitamin D” or “25-hydroxyvitamin D” is used in the context of “vitamin D binding proteins” or “25-hydroxyvitamin D binding proteins,” which are capable of binding with different forms of vitamin D, such as 25-hydroxyvitamin D<sub>2</sub> and 25-hydroxyvitamin D<sub>3</sub>. “Vitamin D<sub>3</sub>” or “25-hydroxyvitamin D<sub>3</sub>” is the actual compound used to measure the amount of “vitamin D binding proteins” or “25-hydroxyvitamin D binding proteins” in a urine sample. The amount of “25-hydroxyvitamin D<sub>3</sub> binding activity” is an indicator of the amount of “25-hydroxyvitamin D binding proteins” in the urine.

Finally, the Examiner alleges that “[c]laim 10 still does not draw a direct correlation between the “calculating” and the “determine salt sensitivity” and Claim 12 offers no reference to salt sensitivity at all” (page 2 of the Office Action). Claim 10 has been canceled. Claim 12 has been amended to address the Examiner’s concerns. New Claim 14 has been added as suggested by the Examiner.

Applicants respectfully submit that the amendments obviate the grounds of the rejection. Withdrawal of the rejection to Claims 7, 11 and 12 under 35 U.S.C. § 112, second paragraph, is respectfully requested. Claims 8-10 have been canceled. Rejection to these claims is now moot.

### **Claim Rejections Under 35 U.S.C. § 103**

Claim 13 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over DeLuca et al. (U.S. Patent No. 4,269,777, hereinafter DeLuca) and Norman et al. (U.S. Patent No. 3,772,150, hereinafter Norman) in view of Garman (U.S. Patent No. 6,054,282), Blume (U.S. Patent No. 6,010,861) and Cook (U.S. Patent No. 5,989,854). Applicants respectfully traverse the rejection.

To establish a *prima facie* case of obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). “All words in a claim must be considered in judging the patentability of that claim against the prior art.” *In re Wilson*, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970). Further, the key to supporting any rejection under 35 U.S.C. § 103 is the clear articulation of the reason(s) why the claimed invention would have been obvious. The Supreme Court in *KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385, 1396 (2007) noted that the analysis supporting a rejection under 35 U.S.C. § 103 should be made explicit. The Federal Circuit has stated that “rejections on obviousness cannot be sustained with mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.” *In re Kahn*, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006). See also *KSR*, 82 USPQ2d at 1396 (quoting Federal Circuit statement with approval).

Claim 13 of the instant application is directed to a test kit comprising: radiolabeled 25-hydroxyvitamin D<sub>3</sub>; unlabeled 25-hydroxyvitamin D<sub>3</sub>; and charcoal, wherein said kit does not contain antibodies.

In contrast, De Luca generally describes isotopically labeled vitamin D compounds. Norman generally describes a method of making a rapidly acting polar metabolite of vitamin D. Garman generally describes a receptor binding assay. Blume generally describes a method using antibodies as screening reagent. Cook generally describes vessels and multiwell plates having a scintillant base. None of the cited references teach or suggest a test kit comprising charcoal, as recited in Claim 13. Accordingly, Claim 13 is patentable over De Luca, Norman, Garman, Blume and Cook, because the cited references, individually or in combination, fail to teach or suggest all claim limitations.

In view of the foregoing, Applicants respectfully submit that the grounds for these rejections have been obviated and withdrawal of the 35 U.S.C. §103 rejection is respectfully requested.

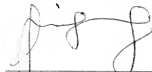
## CONCLUSION

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action and, as such, the present application is in condition for allowance.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of the application, the Examiner is invited to contact Applicants' counsel, Ping Wang (Reg. No. 48,328), at 202.662.3042.

Respectfully submitted,

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A handwritten signature in black ink, appearing to read 'Ping Wang', is written over a horizontal line.

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Attachment: Pages 2 and 7 of the Specification

activity, characteristic of salt-induced hypertension, were reported to have significantly lower plasma 25-OHD concentrations compared with normotensive elderly and young females. Black Americans have a higher rate of salt-sensitive than white Americans, and a higher rate of hypertension. Black Americans have been shown to have significantly lower mean plasma 25-OHD concentrations compared with white Americans in several studies involving both males and females and in a report based on the third National Health and Nutrition Examination Survey (NHANES III, 1988-91). The NHANES III study comprised 18,875 adolescents and adults. Prevalence of low 25-OHD values (less than 10 ng/ml) was greater in non-Hispanic blacks than in non-Hispanic whites.

Low levels of 25-OHD in black Americans have been ascribed, in part, to reduced epidermal vitamin D photosynthesis associated with high melanin skin content. The lower mean plasma 25-OHD concentrations found for black subjects in the studies might also be affected by lower plasma 25-OHD concentrations in a subset of salt-sensitive black subjects with borderline or moderately high blood pressure. In a study of matched pre-menopausal females without history of hypertension, mean serum concentration of 25-OHD was slightly, but not significantly, lower in the 70 black subjects compared with the 67 white subjects. Young Dahl S rats fed a low salt diet exhibit plasma 25-OHD concentrations slightly, but not significantly, lower than that of R rats.

Based on the blood pressure change in response to a salt load, a previous report found 181 and 37% salt sensitivity for 18-23 year-old Caucasian and African-American subjects, respectively. Another report found 22% prevalence of salt sensitivity in 140 African-American adolescents.

#### Summary of the Invention:

It is the purpose of this invention to find an economical, non-intrusive and rapid means of identifying salt-sensitive persons who may have or can be expected to develop

Prospect, IL). Duplicate incubations were made for each rat. Binding in the presence of 200 fold excess unlabeled 25-OHD<sub>3</sub> (non-specific binding) was subtracted from the total binding to obtain specific binding.

**Protein:** Urinary protein was measured by the. biconchonic acid protein assay (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instruction, using an automated plate reader.

**Statistical analyses:** A mean  $\pm$  SEM was calculated for each group. Statistical significance was evaluated using two-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test (SigmaStat, SPSS, Inc., Chicago, IL.) The Mann-Whitney test was used for baseline comparisons, when rats were fed a non-purified diet.

#### Results:

In the set of studies, female S rats excreted  $0.03 \pm 0.01$  nmole 25-OHD/24 hours into urine at week 2 of low salt intake, whereas secretion by female R rats was non-detectable. Female S rats excreted  $0.26 \pm 0.04$  nmole 25-OHD/24 hours at week 2 of the high salt intake. This level was five times that of female R rats at week 2 of high salt intake ( $0.05 \pm 0.02$  nmol/24 hours) and nine times that of S rats at week 2 of low salt intake.

The calculated 25-hydroxyvitamin D binding activity in the 24 hour urine of female S rats was  $79 \pm 11$  pmol/hour at week 2 of high salt intake, two times that in the urine of S rats at week 2 of low salt intake ( $33 \pm 10$  pmol/hour) and greater than 35 times that in the urine of female R rats at week 2 of low ( $1.5 \pm 1.4$  pmol/hour) of high salt intake ( $2.1 \pm 1.5$  pmole/hour).

Urinary protein of S rats was significantly affected by salt ( $P<0.001$ ) and by duration of high salt intake ( $P=0.05$ ). Urinary protein of S rats was significantly higher than that of R rats during low ( $P=0.02$ ) and high ( $P<0.001$ ) salt intake. Urinary 25-OHD was directly correlated ( $r=0.96$ ,  $P<0.02$ ) with